Thiol Reactivity Analyses to Predict Mammalian Cell Cytotoxicity of Water Samples

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Detailed Procedure of NAC Thiol Reactivity Assay. The preparation of the reagents for the NAC Reactivity Assay are listed below.

1. **Tris buffer, 200 mM, pH 8.** In a beaker, add 230 mL distilled water, 4.44 g Tris HCL and 2.65 g Tris Base, mix, adjust to 250 mL using a volumetric flask. Pour into a reagent bottle, sterilize by autoclaving.
2. **Potassium phosphate buffer, 100 mM with 0.1 mM EDTA, pH 8.** Separately prepare 100 mL each of 1 M dibasic potassium phosphate and 1 M monobasic potassium phosphate each in distilled water using volumetric flasks. Prepare a 200 mM EDTA solution in distilled water. For the 100 mM potassium phosphate buffer for the assay mix 9.4 mL of the 1 M dibasic potassium phosphate solution plus 0.6 mL of the monobasic potassium phosphate solution with 50 μL of 200 mM EDTA. Add distilled water to 100 mL using a volumetric flask, autoclave.
3. **Ellman's reagent.** Prepare a 1 mM DTNB solution (5, 5'-dithiobis (2-nitrobenzoic acid), (DTNB) in a 100 mM potassium phosphate buffer with 0.1 mM EDTA, pH 8 for a final concentration of 1 mM DTNB. Filter-sterilize the solution store for no more than 4 months at 4°C in the dark.
4. ***N*-Acetyl-*L*-cysteine (NAC) reagent.** Prepare a 100 mM stock solution of NAC in 200 mM Tris buffer, pH 8 and stored at 4°C. The NAC solution should be stored no longer than 1 month.
5. **Maleimide reagent.** Prepare a 100 mM stock solution of 2,5-Pyrroledione (maleimide) in absolute ethanol. Store at 4°C. Maleimide is used as a positive control for the assay.
6. **NAC Thiol Assay form.** A general assay form based on a 96-well microplate was developed for conducting the assay and is presented below.

In general a test sample is reacted with NAC for 20 min in a volume of 50 µL followed with the addition of 50 µL DTNB for resolution at A_{412}. Each microplate contained a concurrent negative control (Figure S1, column 1), positive control (Figure S1, wells 12E-12H)), sample concentrations, and their corresponding blanks. For the negative control, each well contained 40 µL Tris buffer pH 8, 10 µL of 2 mM NAC, and 50 µL of 1 mM DTNB. For the positive control, each well contained 38 µL Tris buffer pH 8, 10 µL of 4 mM NAC, 2 µL of 10 mM maleimide and 50 µL of 1 mM DTNB. For the treatment groups, each well typically contained 10 µL of 4 mM NAC, 50 µL of 1 mM DTNB, a serial dilution of the concentrated water XAD extract and Tris buffer at pH 8. The total volume of the sample and Tris buffer was 40 µL. Sample blanks are important because they correct for the background A_{412}. Each corresponding blank well typically contained 50 µL of 1 mM DTNB, an identical volume of sample to which this blank corresponds, and Tris buffer at pH 8 for a total volume of 100 µL, without NAC was added first followed by the test sample (or maleimide positive control) and the NAC was added last. After 20 min incubation on a rocker platform in the dark, 50 µL of 1 mM DTNB was added to quantify the available thiols. Directly after the addition of DTNB, the plate was analyzed at 412 nm on a microplate reader after linear shaking of 10 sec (Figure S1).

Table S1. Form.

NAC THIOL REACTIVITY ASSAY

Date: Experiment №

Test Agent:

Stock Sol:

DIL A*

DIL B*

DIL C*

Microplate Wells	Tris pH8 µL	4 mM NAC µL	Agent µL	DIL	1 mM Ellman's Sol. µL **	Agent Concentration or Concentration Factor
A1-D1	40	10	–	–	50	400 µM NAC Control
A2-D2		10			50	
A3-D3		10			50	
A4-D4		10			50	
A5-D5		10			50	
A6-D6		10			50	
A7-D7		10			50	
A8-D8		10			50	
A9-D9		10			50	
A10-D10		10			50	
A11-D11		10			50	
A12-D12		10			50	
G1-H1	50	0	–	–	50	Corresponding Blank
G2-H2		0			50	Corresponding Blank
G3-H3		0			50	Corresponding Blank
G4-H4		0			50	Corresponding Blank
G5-H5		0			50	Corresponding Blank
G6-H6		0			50	Corresponding Blank
G7-H7		0			50	Corresponding Blank
G8-H8		0			50	Corresponding Blank
G9-H9		0			50	Corresponding Blank
G10-H10		0			50	Corresponding Blank
G11-H11		0			50	Corresponding Blank
G12-H12		0			50	Corresponding Blank

* All sample agents are diluted in Tris buffer (pH 8). Total volume of reaction well = 50 µL. ** After 20 min reaction time while shaking, room temperature add the 50 µL Ellman's reagent. Total volume of wells = 100 µL immediately read plate in Spectra-Max at 412 nm. *** Positive control = 2 µL of a 10 mM maleimide in EtOH. Put positive control in wells E1, E2. (10 µL NAC), 2 µL Maleimide stock solution plus 38 µL Tris buffer. NAC 4 mM: 100 mM NAC stock - 4/100 ×1000 = 40 µL NAC stock + 960 µL Tris buffer. M. Plewa, University of Illinois at Urbana-Champaign, mplewa@illinois.edu

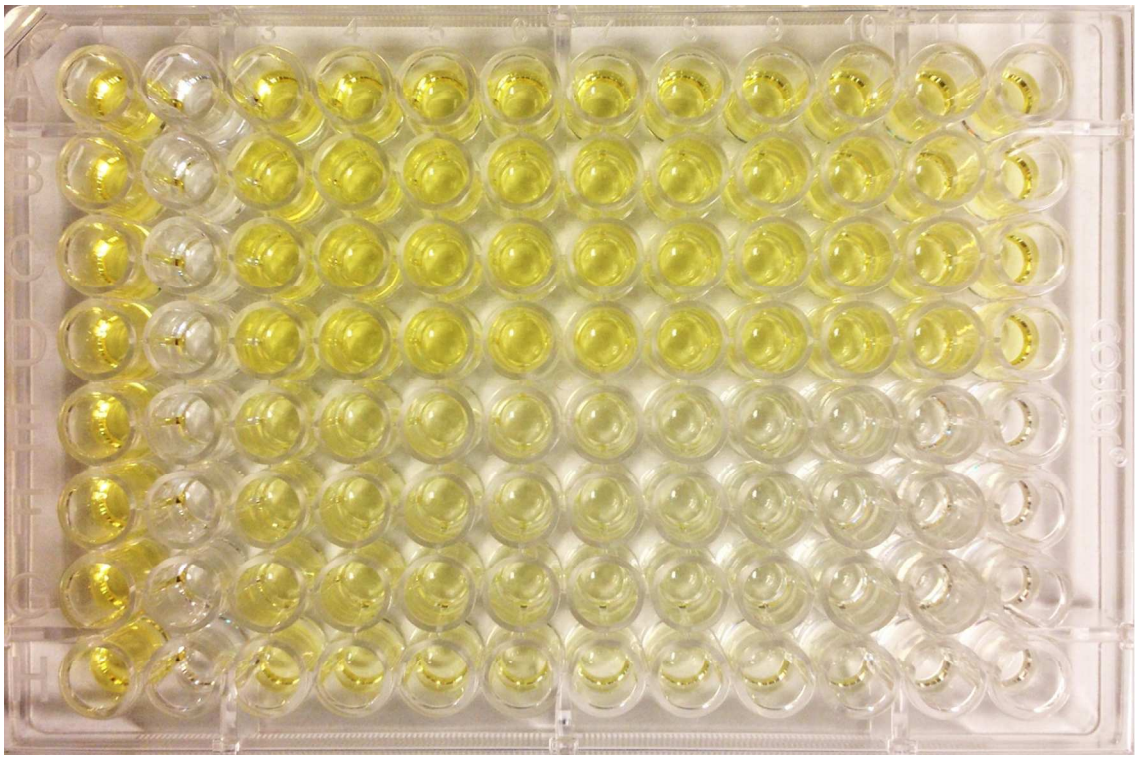

Figure S1. Image of a NAC Thiol reactivity plate illustrating the loss of reacting thiol groups as function of the concentration of a test sample.

Table S2. Calculated LC_{50} values from EC_{50} values. Double click the following table to activate its embedded equations:

Note: Enter the obtained EC50 value from NAC thiol reactivity assay into the						
Type	**Parameter**	**Value**				
Enter	EC50					
Prediction	Predicted a	0.676				
Prediction	Predicted l	-4.95297				
Prediction	Predicted u	6.304974				